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IMMUNOLOGY

Second Edition

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Cover credits
Background: lymph node macrophage attached to an endothelial cell.

Inset: X-ray crystallography of a peptide bound to a human class II MHC molecule, DR1.
Courtesy of J. H. Brown, 1993, *Nature* 364:33.

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been shown to express CD8 and recognize antigen associated with class I MHC, and some functional T_C cells are class II restricted and express CD4. Even the functional classification is not absolute. For example, many T_C cells have been shown to secrete a variety of cytokines and exert effects on other cells comparable to that exerted by T_H cells. The distinction between T_H and T_C cells, then, is not always clear; there can be ambiguous functional activities. However, because these ambiguities are the exception and not the rule, the general description of T helper cells as being $CD4^+$ and class II restricted and of T cytotoxic cells as being $CD8^+$ and class I restricted is adhered to, unless otherwise specified, throughout this text.

Null Cells

A small group of peripheral-blood lymphocytes, called null cells, fail to express the membrane molecules that distinguish T and B lymphocytes (Figure 3-9c). These cells also fail to display antigen-binding receptors of either the T- or B-cell lineage and therefore lack the attributes of immunologic specificity and memory. One functional population of null cells called *natural killer (NK)* cells are large, granulated lymphocytes; these cells constitute 5–10% of the peripheral-blood lymphocytes in humans.

The natural killer cell was first described in 1976, when it was shown that certain null cells display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense against tumor cells. NK cells can interact with tumor cells in two different ways. In some cases, an NK cell makes direct membrane contact with a tumor cell in a nonspecific, antibody-independent process. Some NK cells, however, express CD16, a membrane receptor for the carboxyl-terminal end of the antibody molecule. These NK cells can bind to antitumor antibodies bound to the surface of tumor cells and subsequently destroy the tumor; this specific process is called *antibody-dependent cell-mediated cytotoxicity.* The exact mechanism of tumor-cell killing by NK cells, the focus of much current experimental study, is discussed further in Chapter 15.

Several lines of evidence suggest that NK cells play an important role in host defense against tumors. For example, in humans, Chédiak-Higashi syndrome—an autosomal recessive disorder—is associated with an absence of NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called *biege* lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumor cells.

Mononuclear Cells

The mononuclear phagocytic system consists of circulating monocytes in the blood and macrophages in the tissues. During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes. Monocytes circulate in the bloodstream for about 8 h, during which time they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages.

Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five- to tenfold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of lytic enzymes, and begins to secrete a variety of soluble factors (Figure 3-10). Macrophages are dispersed throughout the body. Some take up residence in particular tissues becoming *fixed macrophages,* whereas others remain motile and are called *free,* or wandering, *macrophages.* Free macrophages move by amoeboid movement throughout the tissues. Fixed macrophages serve different functions in different tissues and are named to reflect their tissue location. In the liver they are called *Kupffer cells;* in the connective tissues, *histiocytes;* in the lung, *alveolar macrophages;* in the kidney, *mesangial cells,* and in the brain, *microglial cells.*

Macrophages are normally in a resting state, but in the course of an immune response, a variety of stimuli activate macrophages. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated T_H cells, by mediators of the inflammatory response, and by bacterial cell-wall products. One of the most potent activators of macrophages is interferon gamma (IFN-γ) secreted by activated T_H cells. Compared with resting macrophages, activated macrophages have increased phagocytic activity, increased microbicidal activity, increased secretion of inflammatory mediators, and an increased ability to activate T cells. This increased activity enables these cells to more effectively eliminate potential pathogens. In addition, activated macrophages secrete various cytotoxic proteins that help them eliminate a broad range of pathogens, including virus-infected cells, tumor cells, and intracellular bacteria. Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. Thus macrophages and T_H cells exhibit an interacting relationship during the immune response with each facilitating activation of the other.

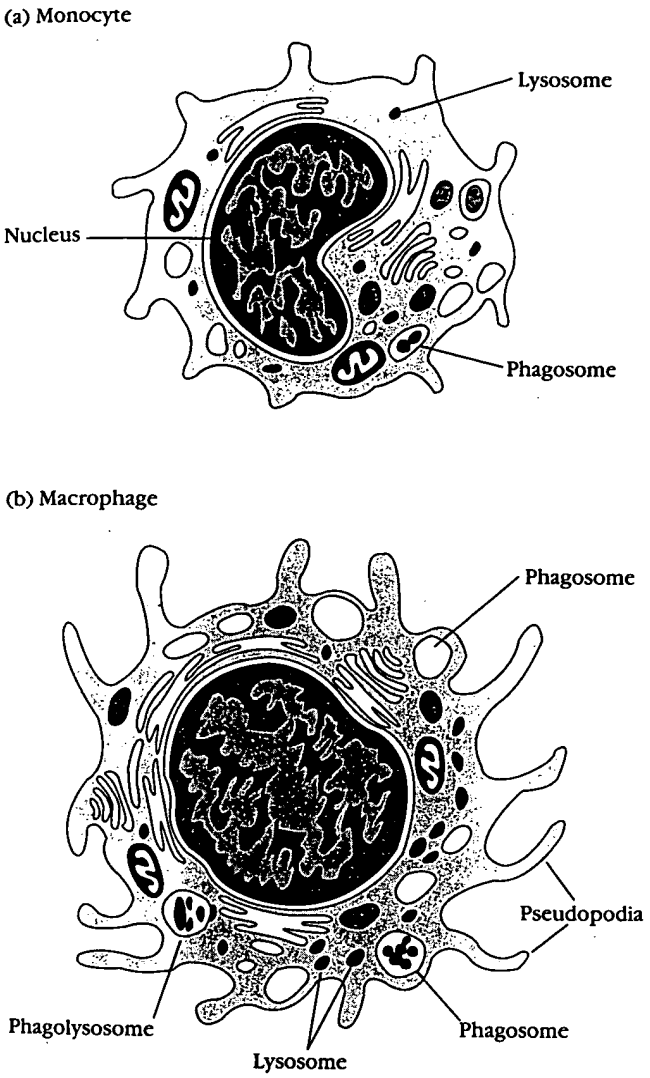

FIGURE 3-10 Drawings showing typical morphology of a monocyte and macrophage. Macrophages are five- to ten-fold larger than monocytes and contain more organelles, especially lysosomes.

Phagocytosis

Macrophages are actively phagocytic cells capable of ingesting and digesting exogenous antigens such as whole microorganisms, insoluble particles, injured and dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called *chemotaxis.* The next step in phagocytosis involves *adherence* of the antigen to the macrophage cell membrane. (Complex antigens, such as whole bacterial cells or viral particles, tend to adhere

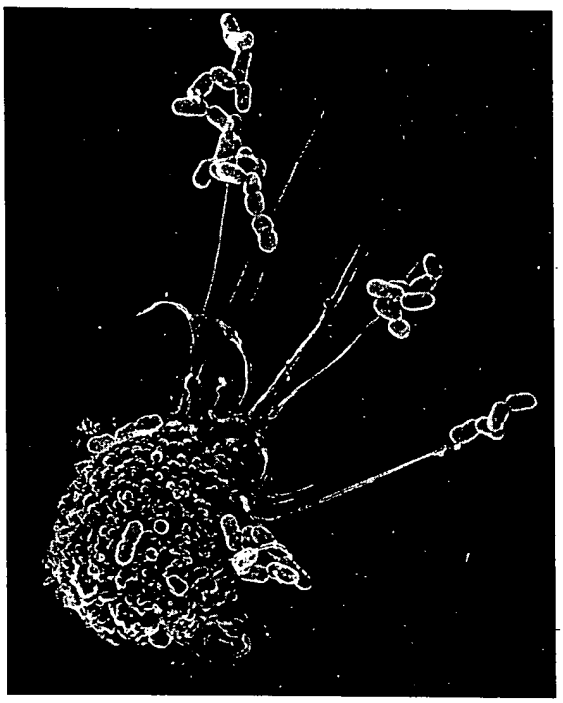

FIGURE 3-11 Scanning electron micrograph of a macrophage. Note the long pseudopodia extending toward and making contact with bacterial cells, an early step in phagocytosis. [Photograph by Lennart Nilsson; courtesy of Boehringer Ingelheim International GmbH.]

well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed.) Adherence induces membrane protrusions, called *pseudopodia,* to extend around the attached material (Figure 3-11). The pseudopodia fuse enclosing the material within a membrane-bound structure called a *phagosome,* which then enters the endocytic processing pathway. In this pathway, a phagosome moves toward the cell interior, where it fuses with a *lysosome* to form a *phagolysosome.* Lysosomes contain hydrogen peroxide, oxygen free radicals, peroxidase, lysozyme, and various hydrolytic enzymes, which digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called *exocytosis* (Figure 3-12).

The phagocytic rate can be increased substantially in the presence of *opsonins,* which are molecules that bind to antigen and to macrophages. The macrophage membrane possesses receptors for certain classes of antibody and certain complement components. When an antigen (e.g., a bacterium) is coated with the appropriate antibody or complement component, it binds more readily to the macrophage membrane; as a result,

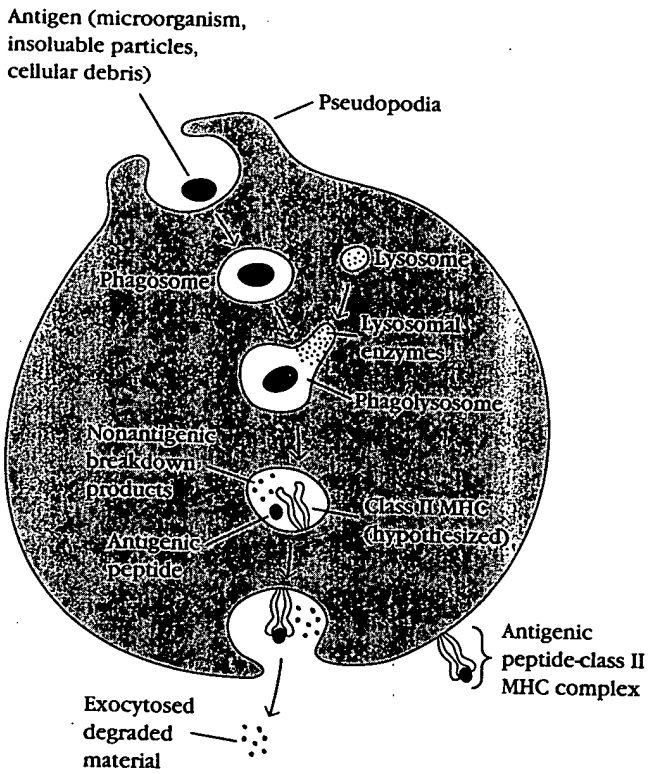

FIGURE 3-12 Phagocytosis and processing of exogenous antigen by macrophages. Following adherence of antigen to the macrophage membrane, long pseudopodia surround the attached material. Fusion of pseudopodia draws the material into the cell, forming a membrane-bound phagosome. Fusion of the phagosome with a lysosome produces a phagolysosome and results in release of the lysosomal contents. Most of the products from digestion of the ingested material are exocytosed. Some of the digested peptides are thought to interact with class II MHC molecules. The resulting antigenic peptide–class II MHC complexes then move to the cell surface where they are presented to T_H cells.

phagocytosis is enhanced. Thus antibody and complement function as opsonins; the entire process is called *opsonization.* In one study the rate of phagocytosis of an antigen was 4000-fold higher in the presence of specific antibody to the antigen than in its absence.

Antimicrobial and Cytotoxic Activities

A number of antimicrobial and cytotoxic substances produced by activated macrophages are responsible for the intracellular destruction of phagocytosed microorganisms (Table 3-4). In addition, these toxic substances can be released from macrophages to mediate potent antitumor activity. The toxic effects of these substances involve both oxygen-dependent and oxygen-independent mechanisms.

OXYGEN-DEPENDENT KILLING MECHANISMS. Activated phagocytes produce a number of *reactive oxygen intermediates* (ROIs) and *reactive nitrogen intermediates* (RNIs) that have potent antimicrobial activity. During phagocytosis a metabolic process known as the respiratory burst occurs in activated macrophages. This process results in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion ($O_2^{\cdot -}$), a reactive oxygen intermediate that is extremely toxic to ingested microorganisms.

$$NAD(P)H + 2O_2 \rightarrow 2O_2^{\cdot -} + NAD(P) + H^+$$

The superoxide anion also generates other powerful oxidizing agents including hydroxyl radicals ($OH^{\cdot}$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2). As the lysosome fuses with the phagosome, myeloperoxidase together with a halide ion act on the hydrogen

TABLE 3-4 MEDIATORS OF ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF MACROPHAGES AND NEUTROPHILS

Oxygen-dependent killing	Oxygen-independent killing
Reactive oxygen intermediates	Defensins
$O_2^{\cdot -}$ (superoxide anion)	Tumor necrosis factor (macrophage only)
$OH^{\cdot}$ (hydroxyl radicals)	Lysozyme
$^1O_2$ (singlet oxygen)	Hydrolytic enzymes
H_2O_2 (hydrogen peroxide)	
HOCl (hypochlorous acid)	
NH_2Cl (monochloramine)	
Reactive nitrogen intermediates	
NO (nitric oxide)	
NO_2 (nitrogen dioxide)	
HNO_2 (nitrous acid)	

peroxide to produce longer-lived oxidants, including hypochlorite, which are toxic.

When macrophages are activated with bacterial cell-wall lipopolysaccharide (LPS) or muramyl dipeptide (MDP) together with a T-cell–derived cytokine (IFN-γ), they begin to express high levels of nitric oxide synthetase, which oxidizes L-arginine to yield citrulline and a reactive radical, nitric oxide (NO). Although NO itself has potent antimicrobial activity, it can combine with the superoxide anion (O_2^-) to yield even more potent antimicrobial substances. Recent evidence suggests that much of the antimicrobial activity of macrophages against bacterial, fungal, helminthic, and protozoal pathogens is due to NO and NO-derived substances.

OXYGEN-INDEPENDENT KILLING MECHANISMS. Activated macrophages contain lysozyme and hydrolytic enzymes whose degradative activities do not involve oxygen. A group of antimicrobial and cytotoxic peptides, commonly known as *defensins,* also are present in activated macrophages. These molecules are cysteine-rich cationic peptides of 29–35 amino acid residues. Each peptide contains six invariant cysteines, which form three intramolecular disulfide bonds. The disulfide bond between the amino-terminal and carboxy-terminal cysteine forms a circular molecule that is stabilized by the other two disulfide bonds into a folded triple-stranded β-sheet configuration. These circularized defensin peptides have been shown to form ion-permeable channels in bacterial and mammalian cell membranes. Defensins can kill a variety of bacteria including *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa,* and *Hemophilus influenzae.*

RESISTANT PATHOGENS. Most phagocytosed microorganisms are killed as the contents of the lysosome are released into the phagosome. Some microorganisms, however, can survive and multiply within macrophages. These intracellular pathogens include *Listeria monocytogenes, Salmonella typhimurium, Neisseria gonorrhoea, Mycobacterium avium, Mycobacterium tuberculosis, Mycobacterium leprae, Brucella abortus,* and *Candida albicans.* Some of these pathogens prevent lysosome-phagosome fusion and proliferate within phagosomes; others have cell-wall components that render them resistant to the contents of lysosomes; and still others survive by escaping from phagosomes and proliferating within the cytoplasm of infected macrophages. These intracellular pathogens, which have developed a clever defense against the nonspecific phagocytic defense system, are shielded from a specific immunologic response. A unique cell-mediated immunologic defense mechanism, called delayed hypersensitivity, combats such pathogens; this mechanism is discussed Chapter 15.

Antigen Processing and Presentation

Not all of the antigen ingested by macrophages is degraded and eliminated by exocytosis. Experiments with radiolabeled antigens have demonstrated the presence of labeled antigen components on the macrophage membrane after most of the antigen has been digested and eliminated. As discussed in Chapter 1, phagocytosed antigen is degraded within the endocytic processing pathway into peptides that associate with class II MHC molecules; these peptide–class II MHC complexes then move to the macrophage membrane (see Figure 3-12). This presentation of antigen is a critical requirement for the activation of T_H cells, a central event in the development of both humoral and cell-mediated immune responses. The processing and presentation of antigen are examined in detail in Chapter 10.

Secretion of Factors

A number of important proteins central to development of immune responses are secreted by activated macrophages (Table 3-5). These include interleukin 1

TABLE 3-5 SOME FACTORS SECRETED BY ACTIVATED MACROPHAGES

Factor	Function
Interleukin 1 (IL-1)	Induces activation of T_H cells following interaction with antigen-MHC complexes; promotes inflammatory response and fever
Complement proteins	Promote elimination of pathogens and inflammatory response
Hydrolytic enzymes	Promote inflammatory response
Interferon alpha (IFN-α)	Activates cellular genes resulting in the production of proteins that confer an antiviral state on the cell
Tumor necrosis factor (TNF-α)	Kills tumor cells
Interleukin 6 (IL-6) GM-CSF G-CSF M-CSF	Promote inducible hematopoiesis

(IL-1), which acts on T_H cells and provides a costimulatory signal required for activation following antigen recognition. Interleukin 1 also acts on vascular endothelial cells, thus influencing the inflammatory response, and affects the thermoregulatory center in the hypothalamus, leading to the fever response.

Activated macrophages secrete a variety of other factors involved in the development of an inflammatory response. These include a group of serum proteins, called *complement,* that assist in the elimination of foreign pathogens and in the ensuing inflammatory reaction. The ***hydrolytic enzymes*** contained within their lysosomes can also be secreted by activated macrophages. The buildup of these enzymes within the tissues contributes to the inflammatory response and can, in some cases, lead to extensive tissue damage. Activated macrophages also secrete soluble factors, such as *tumor necrosis factor α (TNF-α),* that can kill a variety of cells. The secretion of these *cytotoxic factors* has been shown to contribute to tumor destruction by macrophages. Finally, as discussed earlier, activated macrophages secrete a number of cytokines that stimulate inducible hematopoiesis.

Granulocytic Cells

The granulocytes are classified as *neutrophils, eosinophils,* or *basophils* on the basis of cellular morphology and cytoplasmic staining characteristics (Figure 3-13). The neutrophil, which has a granulated cytoplasm that stains with both acid and basic dyes, is often called a *polymorphonuclear leukocyte* for its multilobed nucleus. The eosinophil has a bilobed nucleus and a heavily granulated cytoplasm that stains with the acid dye eosin Y (hence its name). The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not. Neutrophils, which constitute 50–70% of the circulating white blood cells, are much more numerous than eosinophils (1–3%) or basophils (<1%).

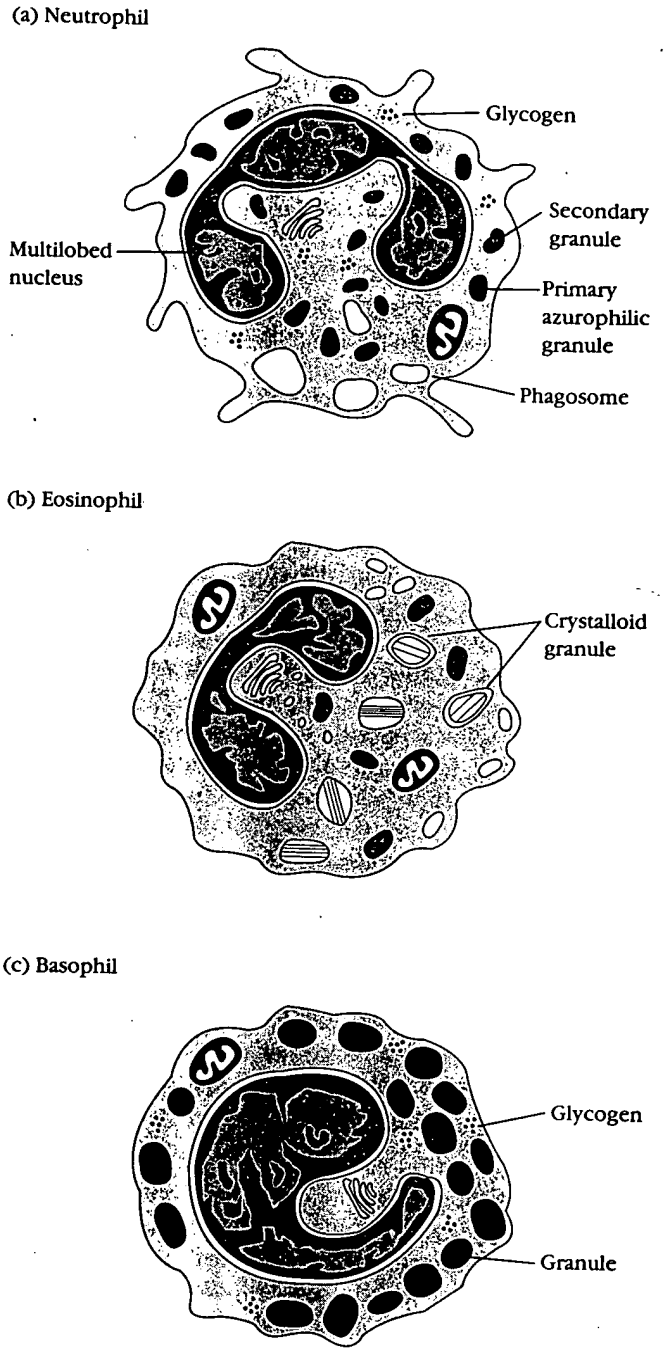

FIGURE 3-13 Drawings showing typical morphology of granulocytes. Note differences in the shape of the nucleus and in the number and shape of cytoplasmic granules.

Neutrophils

Neutrophils are produced in the bone marrow during hematopoiesis. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues where they have a 3-day life span. In response to many types of infections the bone marrow releases increased numbers of neutrophils. The increased numbers of circulating neutrophils, called *leukocytosis,* is used medically to indicate the presence of an infection. Neutrophils generally are the first cell to arrive at a site of inflammation. Observation of neutrophil migration reveals that the cell first adheres to the vascular endothelium, then penetrates the gap between the endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces (this process is discussed in a later section). A number of substances generated in an inflammatory reaction serve as